Platform: Protein Assemblies

193-Plat
The Cooperative Assembly of IFI16 Filaments on dsDNA Provides Insights into Host Defense Strategy

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Whether host DNA-receptors have any capacity to distinguish self from nonself at the molecular level is one of the foremost questions in the innate immunity of mammals. By using quantitative assays and electron microscopy, we show that cooperatively assembling into filaments on dsDNA may serve an integral mechanism by which human interferon inducible protein 16 (IFI16) engages foreign DNA. IFI16 is essential for defense against a number of different pathogens, and its aberrant activity is also implicated in several autoimmune disorders such as Sjögren's syndrome. IFI16 cooperatively binds dsDNA in a length-dependent manner and clusters into distinct protein filaments even in the presence of excess dsDNA. Consequently, the assembled IFI16•dsDNA oligomers are clearly different from the previously proposed noninteracting entities resembling beads on a string. The isolated DNA-binding domains of IFI16 engage dsDNA without forming filaments and with weak affinity, and it is the non-DNA binding pyrin domain (PYD) of IFI16 that drives the cooperative filament assembly. The surface residues on the PYD that mediate the cooperative DNA-binding are conserved, suggesting that related receptors use a common mechanism. These results suggest that IFI16 clusters into signaling complexes in a switch-like manner, and that it may use the size of naked dsDNA as molecular ruler to distinguish self from nonself.

194-Plat
A Helical Transport Mechanism for Type III Secretion

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Gram-negative pathogenic bacteria, like Salmonella typhimurium and Shigella flexneri, employ the Type III Secretion System (T3SS) to infect human cells. The T3SS is a large protein secretion channel that assembles to span a ~50nm gap between the bacterial and target cell walls. A key component of the S. typhimurium SPI-1 T3SS is the 80 residue needle subunit PrgI which polymerizes to form a 25 Å wide channel through which proteins are transported. During needle assembly, the PrgI subunits pass through the nascent channel before attaching to the tip.
We have studied the mechanism of PrgI transport using near-atomistic molecular dynamics simulations. We found that the channel's inward facing amino acids and its helical symmetry direct PrgI diffusion along a helical pathway (the i+1 crystallographic axis) with 2.4nm axial displacement per 360 degrees rotation. In vivo assays have shown that mutations of channel residues inhibit the subunit secretion required for needle self-assembly.
Our combined studies evidence that the channel surface plays an active role in substrate secretion, rather than being a passive corridor for linear diffusion. Our evidence of rotation-translation coupling suggests the that the T3S needle might rotate during effector secretion.

195-Plat
Unraveling the Link between Nonlinear Mechanics, Microstructure, and Molecular Packing of Fibrin

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When we cut ourselves, our body's immediate response is to stop the bleeding by repairing the damage to the wall of the blood vessel_forming a ''blood clot''. This is physically carried out by forming a network of semiflexible fibrin fibers, which bind together red blood cells and platelets, thus effectively plugging the hole that results from the injury. Recent works have shown that fibrin exhibits extraordinary material properties: it can be stretched up to 5 times its original length and it can stiffen more than 100 fold in the process. Unraveling the biophysical mechanisms behind these phenomena not only can help us better understand how our body maintains haemostasis, but also can provide useful design principles for (bio)materials.
In this work, we relate the nanoscale polymerization kinetics to the microscale fiber and network structure, and to the macroscale rheological properties of fibrin. We identified distinct temporal stages in which fibrils aggregate laterally to form floppy fibers, followed by slow compaction of the fibril bundles. Furthermore, we show a direct correlation between the slow formation of high-molecular-weight chain oligomers with a slow decrease in fiber diameter and a concomitant slow increase in clot stiffness. A comparison with theoretical model of bundled semiflexible polymer networks reveals that cross-linking enhances the tightness of coupling between protofibrils within fibers. This compaction leads to the stiffening of fibers, and thus underlies the stiffening of clots. Strikingly, the stiffening effect becomes negligible when the samples are subjected to a large mechanical deformation, suggesting that the mechanics of highly stressed clots is governed by intrinsic fibril stretching. Together, our work provides a detailed biophysical picture explaining how the hierarchical structure of fibrin is interconnected with its formation and mechanics at multiple length-scales.

196-Plat
Dimerization of the PTEN Tumor Suppressor and its Structural Characterization by SAXS

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The PTEN tumor suppressor is a dual-specificity phosphatase whose main target is the phosphatidylinositoltriphosphate (PI(3,4,5)P3) pool in the inner plasma membrane. It acts as the PI3K antagonist in the PI3K/Akt signaling pathway that controls cell growth and apoptosis and is the second most frequently mutated protein in human cancers. Because the propensity for tumor formation depends on PTEN dose in a way that is inconsistent with the ''two-hit hypothesis'', it was postulated that PTEN multimers may form the active species of the phosphatase, and recently strong evidence has been presented for the formation of functional PTEN dimers in the cell (Papa et al., Cell 157, 2014, 595). Here, we use SAXS to investigate the multimerization of PTEN in buffer and show that it indeed forms dimers following elution from a size-exclusion column as a monomer. Electron density envelopes for the PTEN monomer and dimer obtained from SAXS could clearly be distinguished and were assigned by placing the PTEN crystal structure which was earlier determined for a truncated protein. In addition, the monomer envelope was validated by μs-long all-atom MD simulations of full-length PTEN. In these simulations, the auto-inhibitory, flexible C-terminal tail associates closely with the PTEN core domains while hopping between different bound conformations. The equilibrium ensemble of the resulting structures is in excellent agreement with the SAXS data. A structure prediction using the Rosetta docking protocol revealed a putative dimer arrangement that fits the dimer envelope derived from SAXS very well and is consistent with neutron reflectometry results for membrane-bound PTEN.

197-Plat
Molecular Simulations of the Capsid Release and Membrane Binding Processes of Flock House Lytic Peptides

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During the infection process, a virus particle encounters membrane barriers, which must be overcome in order for the viral genome to be delivered to the correct cellular location for viral transcription. While enveloped viruses have fusion peptides to facilitate this process, the mechanisms by which non-enveloped viruses cross these membranes barriers is poorly understood. Many non-enveloped virus contain a membrane lytic component to the viral capsid, which is sequestered on the capsid interior until a signal is received to externalize/activate the lytic component. One such model system is the Flock House virus (FHV), which is an animal virus of the nodaviridae family, which infects insects. FHV has a T=3 icosahedral capsid, and autocatalytic cleavage separates the lytic peptide (γ) from each of the 180 subunits. Lysosome leakage experiments have shown acidic conditions are critical for FHV membrane lysis. Using molecular simulation methods we have been investigating three aspects of this phenomena i) γ externalization form the capsid interior, ii) structural

dynamics of γ in solution and iii) the interaction of γ with membranes. The externalization process has been investigated using steered MD simulations (SMD) under differing protonation states to mimic differing pH conditions. SMD simulations under acidic conditions exhibited structural transitions in the capsid distinct from neutral condition simulations. The structural dynamics of γ have been investigated using metadynamics simulations and indicate γ has a low barrier separating helical and disordered states. The influence of γ structure on membrane binding has been investigated using the MARTINI coarse-grained model to calculate binding free energies. These combined studies have provided new structural and thermodynamic insights into the post-entry stages of non-enveloped virus infection.

198-Plat
Reconstitution of Multivalent PDZ Domain Binding to the Scaffold Protein PSD-95 Reveals Ternary-Complex Specificity of Combinatorial Inhibition
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Multidomain scaffold proteins serve as hubs in the signal transduction network. By physically colocalizing sequential steps in a transduction pathway, scaffolds catalyze and direct incoming signals. Much is known about binary interactions with individual domains, but it is unknown whether ''scaffolding activity'' is predictable from pairwise affinities. Here, we characterized multivalent binding to PSD-95, a scaffold protein containing three PDZ domains connected in series by disordered linkers. We used single molecule fluorescence to watch soluble PSD-95 recruit diffusing proteins to a surface-attached receptor cytoplasmic domain. Different ternary complexes showed unique concentration dependence for scaffolding despite similar pairwise affinity. The concentration dependence of scaffolding activity was not predictable based on binary interactions. PSD-95 did not stabilize specific complexes, but rather increased the frequency of transient binding events. Our results suggest that PSD-95 maintains a loosely connected pleomorphic ensemble rather than forming a stereospecific complex containing all components.

199-Plat
Quantifying Protein-Protein Binding Energy and Entropy using Molecular Dynamics Simulations
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Protein-protein interactions (PPIs) represent a critical aspect of a wide range of biological processes, as most proteins need to associate to various binding partners to carry out their functions. While the basic principles of PPIs are generally understood, it is a grand challenge to accurately predict the binding affinity quantitatively for any given complex. Recently, this potential of mean force (PMF)-based methodology was extended to a much larger protein-peptide system, and we accurately determined the absolute binding free energy between the receptor protein and the ligand peptide. We plan to employ our PMF-based methodology to probe effects of mutations in thermodynamics of protein-protein interaction. Furthermore, thermodynamic decomposition of barstar-barnase protein complex association is attempted to quantify not only to probe the effect of mutation on binding free energy but also on binding entropy.

200-Plat
Generation of Electrochemical Gradient from Peptide Self-Assembly
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The nucleating core of the Amyloid β peptide associated with Alzheimer's disease is able to organize into a peptide bilayer maintaining dimensions similar to biological phospholipid membranes. However, the dynamics of the hydrogen-bonded peptides are certainly different from the flexible alkanes of the lipid membranes, and this crystallinity prompted us to consider their dynamics and functional potential. Here we will review these unique surfaces built on cross-β structures of amyloid, and extend that pattern to mixing of positively- and negatively-charged surfaces to give even higher order architectures with high electrochemical potential across the 4 nm membrane. Further, we have developed EFM analyses to map the charge distribution and explored novel energy or electron transduction reactions. Our results show that these peptide membrane scaffolds are dynamic, and their ability to self-organize offers a new opportunity for engineering specific molecular recognition elements into the peptide assemblies and well-ordered materials.

Platform: Member Organized Session: Protein Nanoassemblies and Networks in Bacterial Chemotaxis and other Two-component Signaling Systems

201-Plat
Insights from Phosphorylation Profiling of an Autoregulated Two-Component System
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Connecting biophysical and biochemical parameters determined *in vitro* to understanding and predicting physiological behavior is an important challenge. Bacterial two-component systems have long served as models for investigating fundamental properties of signal transduction. While understanding regulatory mechanisms has benefited greatly from mathematic modeling, a major obstacle to this approach is the lack of quantitative analyses of two-component systems in their native environments, particularly *in vivo* parameters for histidine kinase and phosphatase activities. Measurement of cellular phosphorylation levels combined with mathematic modeling has enabled a phosphorylation profiling approach to investigate whether protein expression levels of the archetype PhoB/PhoR two-component system are optimized to the phosphorylation output profile and how the positive autoregulatory scheme enables wild-type cells to achieve optimal expression levels of PhoB/PhoR in dynamic environments. The PhoB/PhoR system responds to phosphate (Pi) limitation, and different Pi conditions were discovered to have conflicting requirements for optimal protein expression levels. Experimental evidence established that wild-type cells achieve different optimal expression levels via autoregulation under respective Pi conditions. The fitness optimum balances costs of protein production with benefits, which are correlated with the phosphorylation output. Laboratory evolution experiments revealed that cells with different non-optimal levels of PhoB/PhoR all rapidly evolve toward optimal expression levels by acquisition of diverse mutations, demonstrating strong selective pressure for evolutionary tuning of protein expression levels. However, positive autoregulation comes at the cost of delaying the output response. Analysis of promoter architecture and mathematic modeling suggests that a PhoB repressor site within the *phoBR* promoter provides negative feedback that enables acceleration of the response, counterbalancing the delay imposed by positive autoregulation. Thus system architecture appears to be exquisitely evolved to provide protein activities, levels and timing of expression integrated for optimal response output under dynamic and diverse conditions.

202-Plat
Hydrogen Exchange Reveals Differences between Bacterial Chemoreceptor Signaling States
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Bacterial chemotaxis receptors form membrane-bound nanoarrays that sense and adapt to ligands from the environment. These arrays consist of receptors interacting at their cytoplasmic tips with a ''baseplate'' formed by the CheA kinase and CheW coupling protein, ≈300 Å away from the periplasmic ligand binding sites. Receptor methylation at 4 glutamate residues in the cytoplasmic domain mediates adaptation to ongoing stimuli. To determine what ligand- and methylation-induced changes in the receptor cytoplasmic domain control the kinase activity, we have developed (1) methods to reconstitute native-like arrays of receptor cytoplasmic fragments, CheA, and CheW, and (2) a hydrogen exchange mass spectrometry (HDX-MS) method applicable to membrane-bound, multi-protein complexes. HDX-MS comparison of complexes with high and low kinase activity shows that differences localize to two functionally important subdomains of the receptor. Changes in the methylation subdomain that mediates adaptation are complex. The uniform exchange behavior of these peptides in the kinase-off complexes splits roughly in half in the kinase-on complexes, one fraction with slower exchange and the other with extremely rapid exchange (complete in 3 min). For the signaling subdomain that binds